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Research report

Differential distribution of protein kinase C (PKC$\alpha\beta$ and PKCγ) isoenzyme immunoreactivity in the chick brain

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Abstract

Protein kinase C (PKC) is involved in neural plasticity. The phosphorylation of the myristoylated alanine-rich protein kinase C substrate (MARCKS) in the left intermediate and medial hyperstriatum ventrale (IMHV) of the chick brain has been shown previously to correlate significantly with the strength of learning in filial imprinting. The distribution of PKCα,βI,βII and PKCγ in the brain of 1-day-old dark-reared chicks was determined immunocytochemically, using the monoclonal antibodies MC5 and 36G9, raised against purified PKC$\alpha\beta$ and PKCγ, respectively. PKCγ-stained cells were distributed widely in the telencephalon, including all hyperstriatal structures (including the IMHV), the hippocampus, neostriatum, ectostriatum and archistriatum. There were fewer stained cells in the septum and the least cellular staining was in the paleostriatum primitivum. Fluorescent double-labelling with neuron-specific enolase (NSE) and with the glial calcium-binding protein S100 suggested that PKCγ immunoreactivity was present in neurones but not in glia. The distribution of PKC$\alpha\beta$-stained cells was more limited, with staining in the archistriatum, hippocampus and septum but not in the hyperstriatum. However, there was PKC$\alpha\beta$-staining of some fibres in the IMHV (but little elsewhere in the hyperstriatum ventrale), in the neostriatum, paleostriatal complex and the lobus parolfactorius. Double-labelling with NSE and S100 revealed PKC$\alpha\beta$/S100-positive glial cells present in the paleostriatal region only. There was some PKC$\alpha\beta$-staining of putative neurones in the hippocampus, septum and archistriatum. The differential distribution of PKC isoenzymes suggests that in the IMHV some axonal inputs contain PKC$\alpha\beta$ whereas some postsynaptic cells contain the γ form of PKC.

Keywords: Protein kinase C; PKC; Isoenzyme; IMHV; Imprinting; Double-labeling; Neuron-specific enolase; S100; Glial cell

1. Introduction

Protein kinase C (PKC) is a key enzyme for signal transduction. The enzyme is activated by receptor-stimulated turnover of phosphoinositides [5,42,43,53]. Phosphorylation of PKC substrates has been implicated in neural growth and plasticity [46], including hippocampal long-term potentiation (LTP) in the rat ([34,35,41]; see [33] for a review) and imprinting in the chick *Gallus gallus domesticus* [50]. PKC is also involved in active and passive avoidance learning in the rat [36,58] and chick [12], spatial learning in the rat and mouse [36,45,57] and nictitating membrane conditioning in the rabbit [2,44,48].

Sheu et al. [51] have demonstrated that the phosphorylation of one PKC substrate, myristoylated alanine-rich protein kinase C substrate (MARCKS; $M_r = 67$ kDa in chick brain [19,54]) protein is involved in the learning process of filial imprinting [6]. Specifically, Sheu et al. [50] found that there was a significant positive correlation between a measure of the strength of learning and the phosphorylation of pI $\approx$ 5.0 MARCKS, but not pI $\approx$ 4.0 MARCKS, in a specific brain region. The region, the left intermediate and medial hyperstriatum ventrale (IMHV) of the left hemisphere, has been shown to be crucially involved in filial imprinting and is likely to be a site of information storage [21,23,24]. In this region, an increase in the incorpora-

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tion of uracil into RNA [26] and in the length of the postsynaptic density of axospinous synapses was found after imprinting [11,25]. Learning is also associated with a significant increase in the number of *N*-methyl-D-aspartate (NMDA) receptors in the left but not in the right IMHV [37]. The phosphorylation of another PKC substrate, neuromodulin (F1; $M_r = 50$ kDa, cf. [4]) in the left IMHV did not correlate with the strength of learning but was correlated with locomotor activity during imprinting training [50].

There are several subtypes of PKC which have different levels of phosphorylation of F1 and MARCKS [49]. Meberg et al. [40], using in situ hybridization, studied in chick brain the distribution of mRNA of the PKC substrates F1 and MARCKS. There was robust hybridization for F1 and MARCKS expression in the IMHV. However, little is known about the cellular and subcellular localization of PKC isoenzymes in the chick brain. Therefore, we examined the distribution of PKC$\alpha\beta$ and PKCγ in the chick forebrain, with emphasis on the IMHV. We used the monoclonal antibodies MC5 and 36G9, raised against purified PKC$\alpha\beta$ and PKCγ, respectively [15,16,60]. Preliminary results from this study have been reported elsewhere [8].

2. Materials and methods

2.1. Subjects

12 domestic chicks (Ross I) were used, hatched in darkness in an incubator kept at 37.5° C and reared in a dark incubator at 33° C.

2.2. Western immunoblotting

The monoclonal antibodies MC5 (Amersham), 36G9 [15], and the polyclonal antibody C-19 (Santa Cruz Biotechnology) were characterized by Western blot analysis of chick and rat brain total homogenates. Protein samples (100 μg) were submitted to sodium dodecylsulphate polyacrylamide electrophoresis (SDS–PAGE) on a linear gradient (8–15%) using the buffer system of Laemmli [32]. Proteins were transferred to nitrocellulose membranes by Western blotting [13]. After transfer, the nitrocellulose filter was blocked by overnight incubation in phosphate buffer saline (PBS), containing 5% non-fat dry milk powder, and then incubated in PBS-0.05% Tween 20 solution, containing antibodies diluted according to manufacturers' instructions. The blots were then washed 4 × in PBS-0.05% Tween 20, incubated again for 1 h in blocking solution and incubated in ^{125}I-labelled antibodies against mouse or rabbit immunoglobulins and washed 4 × with PBS-0.05% Tween 20. Blots were exposed to x-ray film and developed.

2.3. Tissue preparation

When the chicks were ≈ 24 h old, they each received an i.p. injection of 0.2 ml Sagatal. When deeply anaesthetized, each chick was perfused transcardially with 50 ml buffered saline, followed by 300 ml 1–2% paraformaldehyde + 0–0.05% glutaraldehyde in phosphate buffer (PB; pH 7.4). The brains were removed from the skull and stored overnight in 30% sucrose in

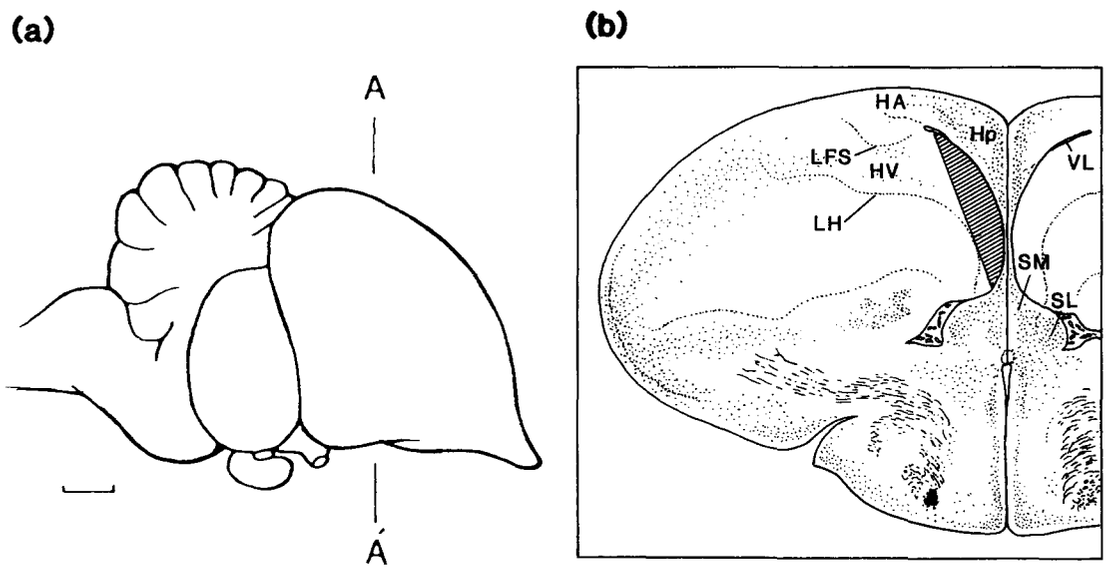

Fig. 1. Schematic drawings of the brain of the domestic chick. a: lateral view of the brain. Vertical lines AA′ indicate the approximate plane of the coronal section of brain in b. Bar = 2 mm. Adapted from [23]. The MHV extends [22] in the antero-posterior plane from A 6.4 to A 9.6 in Kuenzel and Masson [31]. b: simplified diagram of a coronal section of the chick brain at the level of IMHV (approximately A 7.6 in Kuenzel and Masson [31]. The extent in coronal plane of the left IMHV, as removed for biochemical studies (see text), is indicated by the hatched area. Adapted from [1]. Reprinted from [7], with permission.

0.1 M PB. Subsequently, immunostaining was carried out on frozen sections, coronally cut at 20 μm and thaw-mounted on gelatine-coated glass slides.

2.4. Single-labelling

The sections were preincubated for 15 min in 0.1% H_2O_2 in PBS, rinsed in PBS and immersed in 5% normal sheep serum (NSS) in PBS for 30 min to reduce non-specific binding. The sections were then incubated overnight with the first antibody (MC5, a monoclonal mouse anti-PKCαβ IgG or 36G9, a monoclonal mouse anti-PKCγ IgG), diluted 1:100 and 1:200 respectively, in 1% NSS in PBS at 4° C in a wet chamber. After the primary incubation, sections were rinsed in PBS and again preincubated with 5% NSS for 30 min before the secondary incubation step in biotinylated sheep anti-mouse IgG (Amersham), diluted 1:200 in PBS, for 2 h at room temperature. The sections were then rinsed in PBS and incubated in streptavidin-HRP (Zymed) diluted 1:200 in PBS for 2 h at room temperature. Finally, after subsequent rinsing in PBS and Tris buffer, the sections were processed by a diaminobenzidine (DAB)–H_2O_2 reaction (30 mg DAB and 0.01% H_2O_2/100 ml Tris buffer). Control experiments were performed by the omission of the primary antibody (36G9 or MC5).

To measure the percentage of cells that showed nuclear staining for PKCγ in the IMHV, all stained cells were examined in an area of ≈0.5 mm^2 in the middle of the IMHV (Fig. 1). For each of six chicks, cells were counted in two different coronal sections, for both left and right IMHV. The mean number of cells examined per chick was 838.0 ± 38.9 (±S.E.M.) for the left IMHV and 849.2 ± 66.0 for the right IMHV.

2.5. Double-labelling

To examine whether the stained cells were neurones, double-labelling experiments for the study of the coexpression of PKC isoenzymes and neuron-specific enolase (NSE) or S100b, a glial marker [18], in single cells were carried out with fluorescent techniques. The sections were incubated with one of the primary PKC antibodies, as for the single- labelling described above. PKCαβ and PKCγ incubation was followed by phycoerythrin-conjugated goat antimouse IgG (Tago; 1:50, 2 h at room temperature). After completion of the PKC-staining, the sections were incubated overnight at 4° C with rabbit anti-NSE (Sigma, 1:200) or rabbit anti-S100 (Serva, 1:500) followed by fluorescein isothiocyanate (FITC)-conjugated goat antirabbit (Zymed, 1:50, 2 h at room temperature). The glass-mounted sections were coverslipped in a 1:1 mixture of PBS and glycerine, studied and photographed with a Ploemopak Leitz fluorescent microscope with the appropriate filter blocks for FITC and phycoerythrin labels, yielding a green and red fluorescence, respectively. Standard control experiments for both single- and double-labelling were performed by: (1) omission of primary antibodies in the incubation cycle; (2) replacing the primary antibody with normal sera; and (3) primary antibodies incubated with the nonmatching secondary antibodies in case of double-labelling. In all cases, the controls yielded negative results, i.e., the absence of any detectable labelling, excluding the appearance of possible crossreactivity of secondary antisera during the incubation cycle.

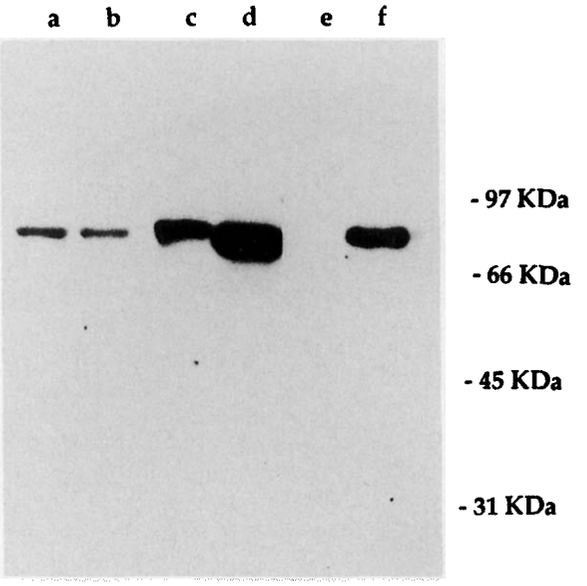

Fig. 2. Western immunoblot analysis of PKC subspecies in chick and rat brain total homogenates. Blots were incubated with antibodies MC5 (a,b), 36G9 (c,d) or C-19 (e,f). Lanes a,c,e, chick brain; lanes b,d,f, rat brain. Molecular weight standards (Bio-Rad) indicated at right margin were: phosphorylase *b* (97 kDa), serum albumin (66 kDa), ovalbumin (45 kDa) and carbonic anhydrase (31 kDa).

3. Results

3.1. Antibody characterization

The monoclonal antibody MC5 recognized protein bands from both chick and rat brain with exactly the same mobility, in the range of 80 KDa (Fig. 2a,b). The monoclonal antibody 36G9 recognized a chick brain protein band with slightly less mobility than that from the rat brain (Fig. 2c,d) whereas the specific polyclonal antibody C-19 against rat, human and mouse PKCγ failed to recognize chick enzyme (Fig. 2e,f).

3.2. Distribution of PKCγ immunoreactivity

PKCγ-positive cells were distributed widely throughout the telencephalon (Fig. 3A). PKCγ-positive

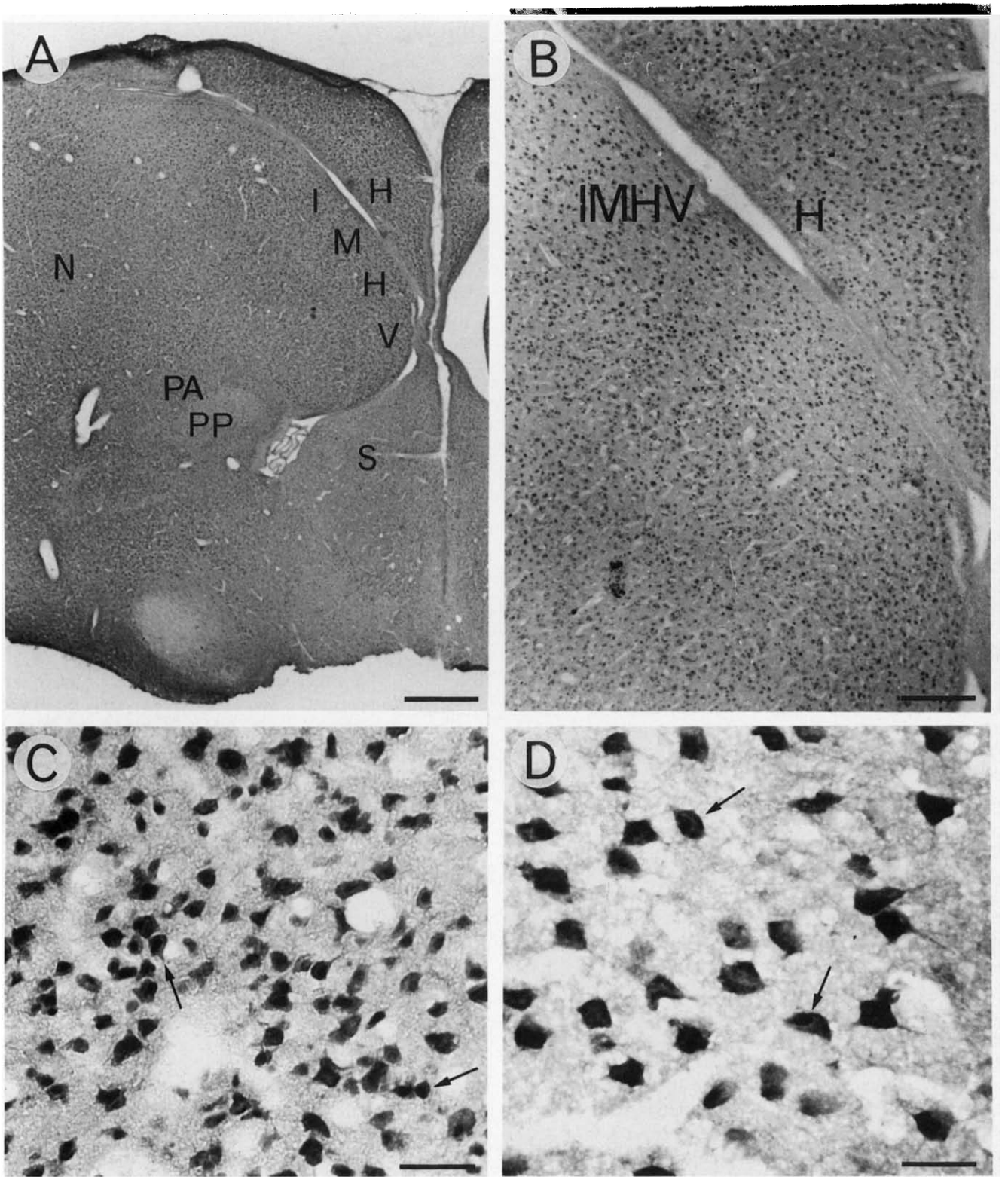

Fig. 3. Distribution of PKCγ immunoreactivity in chick forebrain. A: distribution of PKCγ-positive cells in a coronal section at the level of the IMHV showing neostriatum (N), hippocampus (H), septum (S) and intermediate and medial hyperstriatum ventrale (IMHV). PP, paleostriatum primitivum; PA, paleostriatum augmentatum. B: a higher-power photomicrograph of the section shown in Fig. 1A, depicting the distribution of immunopositive cells in the IMHV and the hippocampus. C,D: high-power photomicrographs of PKCγ-positive cells in the IMHV and the hippocampus, respectively. Most of these cells are polymorphic and display immunoreactivity in the nuclei as well as in the cytoplasm of the cell bodies (arrows). Bar in A, 425 μm; B, 200 μm; C, 45 μm; and D, 35 μm.

cells were typically multipolar, with small and occasionally thick processes emerging from the somata. All hyperstriatal structures, the hippocampus, neostriatum, ectostriatum and archistriatum contained positive cells. There was less dense staining in the septum and the least cellular staining was observed in the paleostriatum primitivum (Fig. 3A). Particularly intense PKCγ immunoreactivity was found in the Purkinje cells of the cerebellum. Immunoreactivity was predominantly present in the cytoplasm of the cell bodies and, to a lesser extent, in processes. A considerable number of PKCγ-positive cells exhibited heavy nuclear staining, notably in the IMHV (arrows in Fig. 3C) and the hippocampus (arrows in Fig. 3D). Faint PKCγ immunoreactivity was observed in the anterior commissure and in the tractus opticus.

In the IMHV, PKCγ-positive cells were distributed homogeneously (Fig. 3A,B). No apparent differences were observed in the nature and degree of staining in the anterior–posterior or the dorso–ventral planes. Most PKCγ-positive cells of the IMHV had a round to oval morphology (Fig. 3C) although some of the IMHV cells along the ventricle had a more elongated appearance. In the IMHVs of the six examined chicks, a mean of 26.4 ± 0.8% (±S.E.M.) of the stained cells showed clear nuclear staining. This percentage did not differ

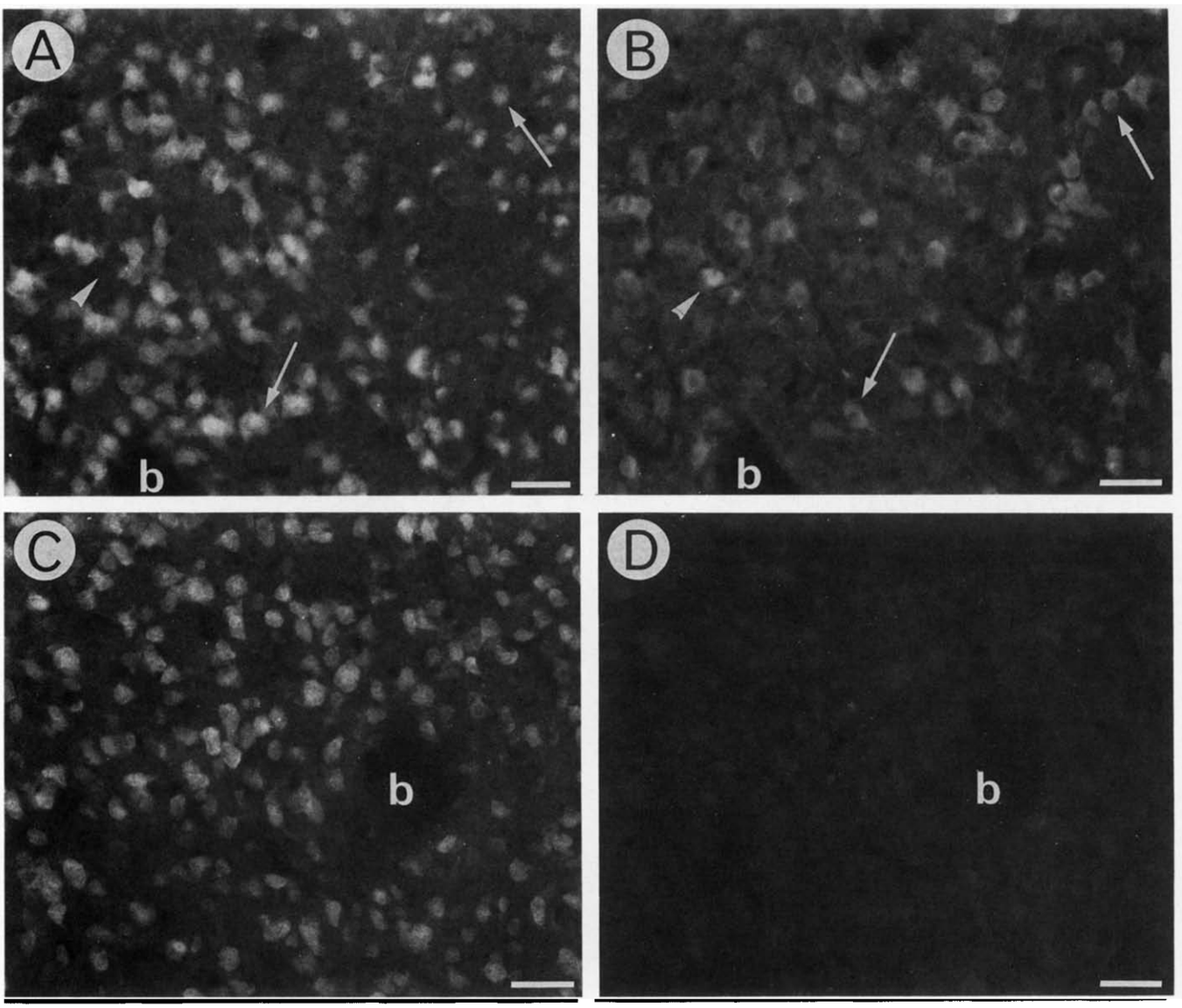

Fig. 4. Fluorescent double-labelling for PKCγ (A,C) and NSE (B) and S100 (D) in the IMHV. A,B: some of the PKCγ-positive cells shown in A are immunolabelled for NSE (B, see arrows). However, the most densely stained NSE-positive neurones appeared to be PKCγ-negative (arrowhead in A,B). C,D: all PKCγ-immunolabelled neurones (C) lack S100 immunoreactivity (D). Blood vessels (b) serve as landmarks for matching fields A with B and C with D, respectively. Bar = 40 μm.

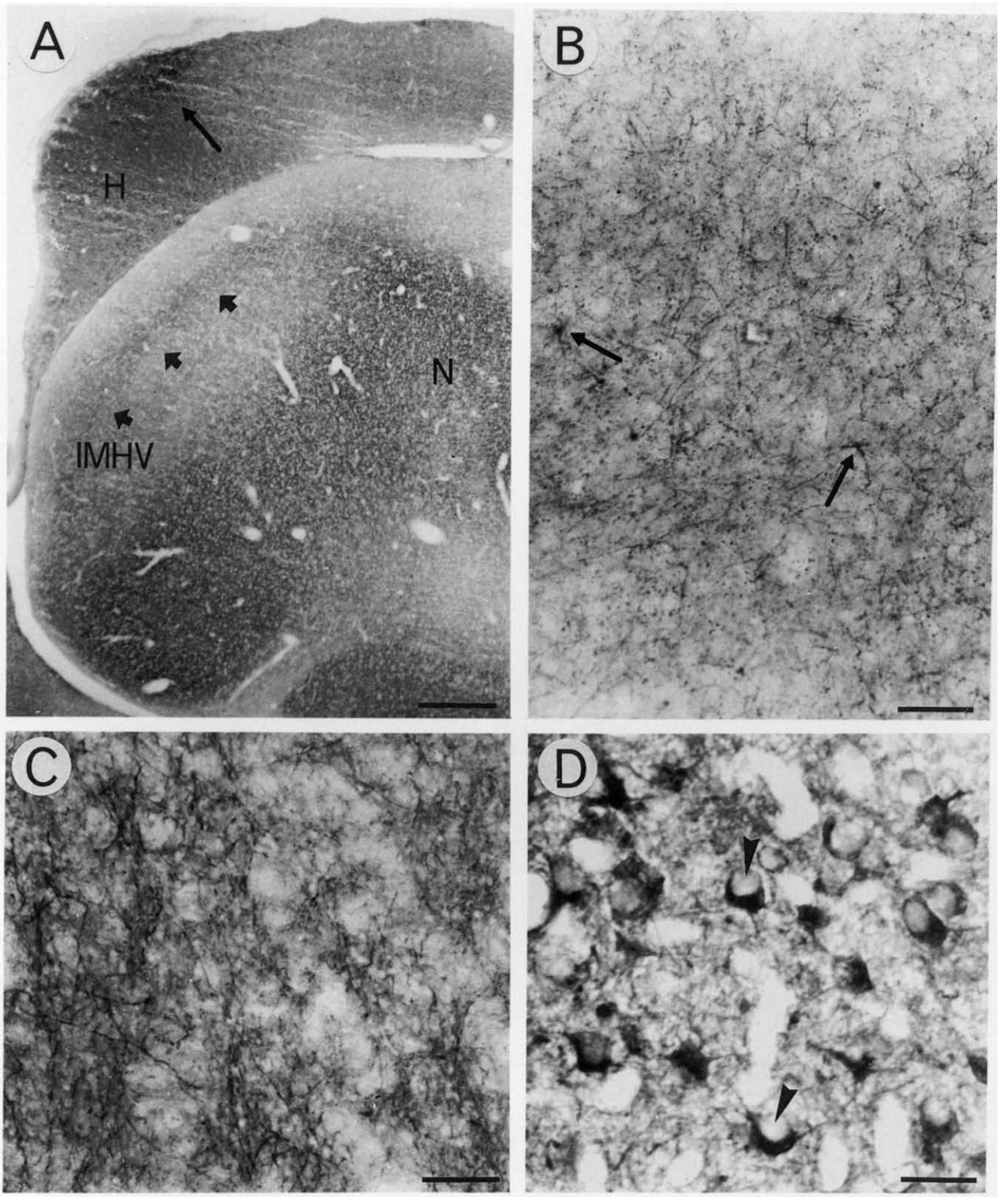

Fig. 5. Distribution of PKC$\alpha\beta$-positive cells and fibres through a coronal section at the level of the IMHV (approximately at A–P level 7.6 in Kuenzel and Masson [31]). A: the neostriatum (N) is characterized by a dense meshwork of PKC$\alpha\beta$-positive fibres. A fibre plexus is found in the IMHV (bold arrows). In the hippocampus (H), a pocket of PKC$\alpha\beta$-positive cells is present (arrow). B,C: high-power photomicrographs of PKC$\alpha\beta$-positive fibres in the IMHV (B) and neostriatum (C). Some small glial-like cells are found in the IMHV (arrows in B). D: PKC$\alpha\beta$-labelled cells in the hippocampus, with large immunonegative nuclei (arrowheads). Bar in A, 400 μm; in B–D, 20 μm.

between the left (26.0%) and right (26.9%) IMHV. No apparent differences in morphology were found between PKCγ-positive cells with respect to the presence of nuclear staining.

The results of the double-labelling experiments, to determine whether the PKCγ-stained cells were neurones, are shown in Fig. 4. PKCγ-positive cells are shown in Fig. 4A,C. Fig. 4B indicates that the staining intensity for NSE varied between cells. Fluorescent double-labelling revealed that some PKCγ-labelled cells were NSE-positive (arrows in Fig. 4A,B) although the most densely NSE-labelled cells were frequently devoid of PKCγ immunoreactivity (arrowhead in Fig. 4A,B). However, all PKCγ-positive cells lacked S100 immunoreactivity (Fig. 4C,D). Furthermore, there was no obvious difference in cell morphology of PKCγ-positive cells that showed clear NSE-staining and those that did not. Only a subset of cells in the IMHV (as well as in other brain regions) expressed the γ form of PKC since some of the NSE-positive cells in the IMHV appeared to be PKCγ-negative (Fig. 4A,B). Taken together, these results suggest that a high proportion, if not all, of the PKCγ-positive cells are neurones; but not all neurones contain PKCγ.

3.3. Distribution of PKCαβ immunoreactivity

The distribution of PKCαβ immunoreactivity differed markedly from that of PKCγ. Dense cellular PKCαβ immunoreactivity was highly regionalized in the chick forebrain. It was present in restricted parts of the hippocampus (Fig. 5A,D), in the archistriatum, cortex piriformis, paleostriatum primitivum and subjacent nucleus intrapeduncularis (Fig. 6A), septum and along the border of the lateral ventricle (paraventricular nucleus, not shown).

Three types of PKCαβ-positive cells could be distinguished based on their size and the intracellular location of the immunoprecipitate. The first type of cells was found in the paleostriatum primitivum and subja-

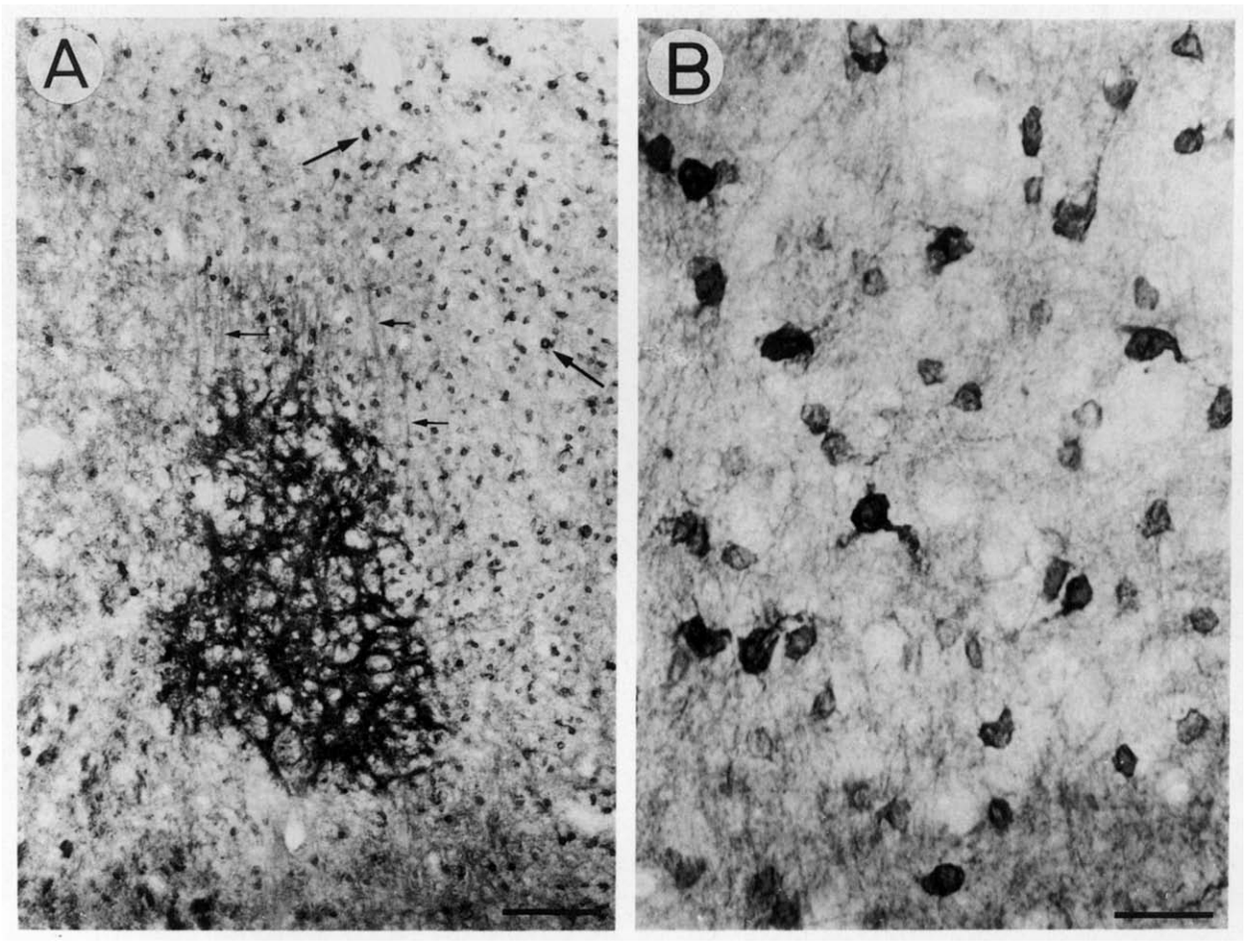

Fig. 6. Distribution of PKCαβ immunoreactivity in the paleostriatal region, comprising the paleostriatum primitivum and nucleus intrapeduncularis. A: a dense fibre plexus (which may be the fasciculus presencephali lateralis or lateral forebrain bundle) and more weakly stained fibres traversing the plane of the section (small arrows) are found in the paleostriatal region. Throughout this region, numerous PKCαβ-positive cells can be seen (large arrows). B: high-power photomicrograph of the PKCαβ-positive cells in an adjacent section, in approximately the same area as where large arrows are in A. Bar in A, 325 μm; in B, 25 μm.

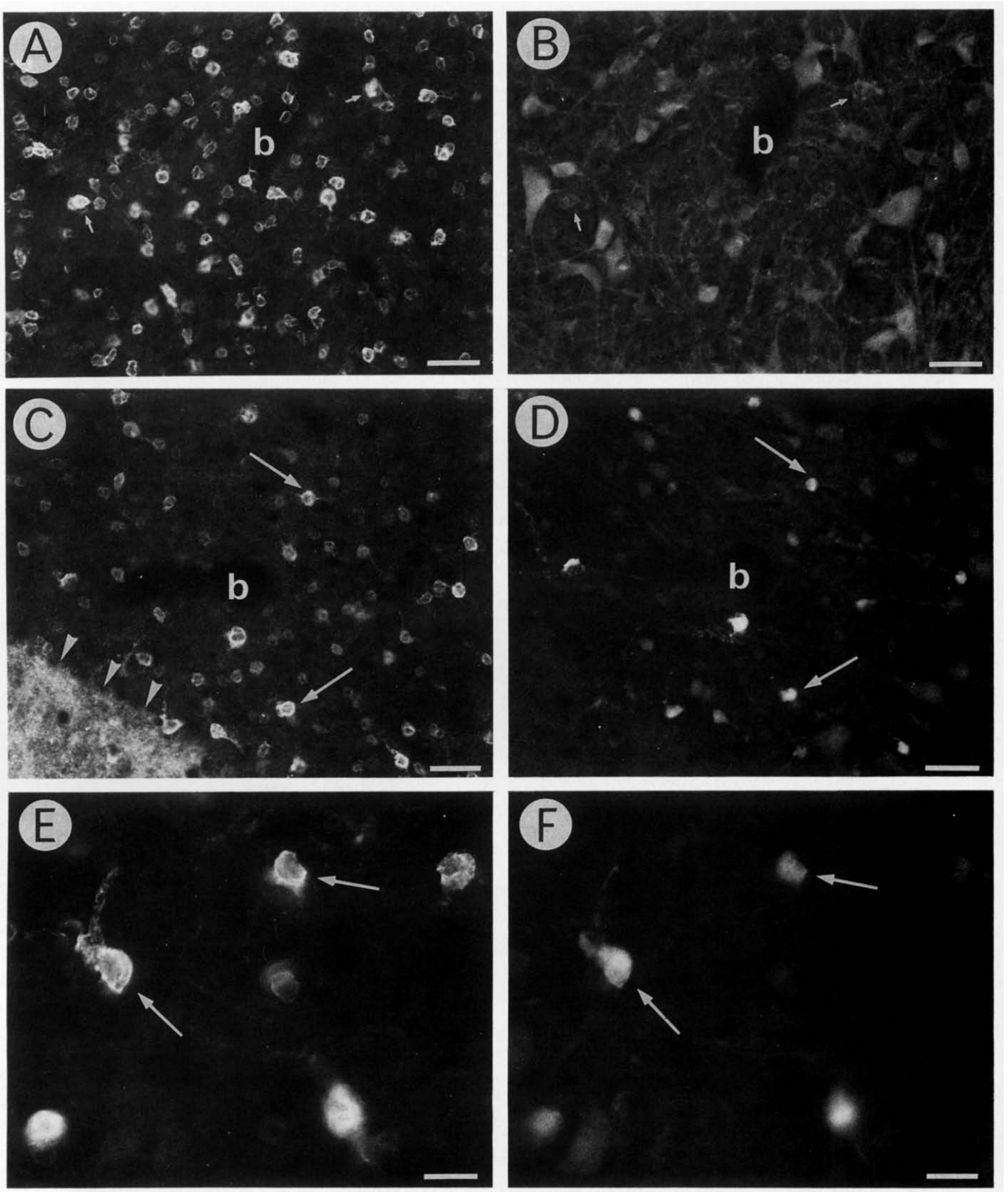

Fig. 7. Fluorescent double-labelling for PKC$\alpha\beta$ (A,C,E) and NSE (B) and S100 (D,F) in the paleostriatal region (Fig. 5). A,B: all PKC$\alpha\beta$-positive cells (A) are NSE-negative (B). Due to strong phycoerythrin fluorescence of the S100 cells (A), some faint yellowish fluorescence is seen under the FITC filter (B; small arrows in A,B) which could easily be distinguished from the green fluorescence of the NSE-positive cells. C,D: all S100-positive cells (D) are intensely stained for PKC$\alpha\beta$ (large arrows). In contrast, cell bodies weakly stained for PKC$\alpha\beta$ generally display low or are even devoid of S100 immunoreactivity. The PKC$\alpha\beta$-positive fibre plexus (arrowheads in C) is S100-negative. E,F: high-power photomicrograph of cells double-labelled for PKC$\alpha\beta$ (E) and S100 (F, see arrows). Blood vessels (b) serve as landmarks to compare field A with B and C with D, respectively. Bar in A,B and C,D = 50 μm; in E,F = 20 μm.

cent nucleus intrapeduncularis (immediately dorsal to the dense fibre meshwork in the middle of Fig. 6A; the regions could not be delineated clearly on the basis of PKC$\alpha\beta$ immunoreactivity). In the soma of these cells, PKC$\alpha\beta$ immunoreactivity was often located close to the cell membrane (Fig. 6B), the cytoplasm then being strikingly devoid of immunoreactivity (Fig. 6B). Cell processes which were clearly stained were observed only occasionally. Fluorescent double-labelling with S100 and NSE revealed that cells in the paleostriatum containing PKC$\alpha\beta$ immunoreactivity were always NSE-negative (Fig. 7A,B) and, therefore, unlikely to be neurones. These cells were frequently S100-positive (Fig. 7C–F), i.e., glial cells. Conversely, *all* S100-positive glial cells displayed PKC$\alpha\beta$ immunoreactivity (Fig. 7C–F). The second type of PKC$\alpha\beta$-immunoreactive cells, found in the hippocampus, archistriatum, cortex piriformis, septum and paraventricular nucleus, showed uniform staining of the cytoplasm and a notable absence of immunoreactivity from the nucleus (e.g. Fig. 5D). Fluorescent double- labelling showed that the PKC$\alpha\beta$-positive cells in the hippocampus were S100-negative. There were no data for NSE/PKC$\alpha\beta$ double-labelling in the hippocampus but the morphology of PKC$\alpha\beta$-positive cells in this structure was not obviously different from that of NSE-positive cells in other regions. These cells are, therefore, likely to be neurones. Cells of the third type were small and widely distributed throughout the brain (including the IMHV) and revealed weak immunostaining to PKC$\alpha\beta$ (arrows in Fig. 5B). These cells, on the basis of their shape and size, may be glia. Occasionally, these cells appeared to be associated with blood vessels. Some endothelial cells immunopositive for PKC$\alpha\beta$ were found also throughout the brain.

Strongly stained fibres were present in the neostriatum (Fig. 5C), paleostriatum primitivum and lobus parolfactorius. The anterior commissure was stained less intensely and numerous PKC$\alpha\beta$-positive glial cells (of the first type, as described above) were embedded within this fibre bundle. There was a band of weakly stained PKC$\alpha\beta$-positive fibres in the main body of the IMHV (centred on approximately A–P 7.6 [31]; Fig. 5A,B) but not in the anterior half of the IMHV (centred on approximately A–P 9.0 [31]). In the dorso–ventral plane, this band was notably restricted to the middle portion of the IMHV (Fig. 5A; see Fig. 1 for the extent of the IMHV). No apparent differences were observed in the anatomical distribution of PKC$\alpha\beta$ between the left and the right IMHV.

4. Discussion

In the present study, we demonstrate region-specific distribution of different PKC subtypes in the brain of the domestic chick. The PKCγ-positive cells frequently stained positive for NSE and were always negative for the glial marker S100. This renders it likely that the vast majority of these cells were neurones. There were also small numbers of S100-negative PKC$\alpha\beta$ cells, probably neurones, in some forebrain regions. These cells may be the source of PKC$\alpha\beta$-positive fibre tracts, seen in the IMHV and elsewhere (see below). PKCγ and PKC$\alpha\beta$ are also localized in some fibre tracts whereas PKC$\alpha\beta$ is additionally present in S100-positive glial cells in the paleostriatal complex, in small S100-negative glial cells throughout the forebrain and in some endothelial cells. There was nuclear staining for PKCγ in some cells throughout the chick forebrain; no nuclear staining was seen for PKC$\alpha\beta$-positive cells.

Differential distribution of PKC isoenzymes has been demonstrated for the rat brain and it has been suggested that these differences reflect PKC subtype-specific functions [43,47,49]. However, little is known about parallel functions of PKC isoenzymes in rat and chick brain and caution should be exercised in comparing PKC subtype functions between species as there may be differences in enzyme structure. For example, the binding characteristics of the monoclonal antibody 36G9 in rat and mouse brain [56–59] are comparable with those in chick brain, with cerebellar Purkinje cells expressing PKCγ most abundantly. In contrast, the polyclonal antibody C-19, specific for human, rat and mouse PKCγ, failed to recognize chicken enzyme on Western immunoblots. In addition, the monoclonal antibody 15G4, raised against purified bovine brain PKCγ [15] failed to show any immunoprecipitation in the chick brain (unpubl. data) whilst in rat brain staining with 15G4 was comparable to that with 36G9. The epitopes of both 36G9 and 15G4 are located on the regulatory subunit of PKCγ, recognizing the 164–197 sequence and the 297–310 sequence, respectively, whereas the epitope for C-19 is in the catalytic part of the enzyme, recognising the 679–697 sequence. These findings indicates that, although similar enzymic forms of PKC are expressed in rat and chick brain, some structural differences may exist between rat and chick PKCγ.

A considerable percentage (26.4% in the IMHV) of PKCγ-positive neurones in the chick brain display strong nuclear staining. In rat brain, PKCγ is known to be present in the nucleus [27]. Cleavage of PKCγ by the proteolytic enzyme calpain results in a constitutively active subunit and a regulatory subunit [29,55]. This regulatory subunit may have some role in cellular function by influencing genomic activation, as the subunit has a DNA-binding motif [30]. 36G9 recognizes the intact PKCγ molecule, as well as its regulatory subunit after cleavage [15,16,58]. Thus, 36G9 can visualize the presence of this subunit in the nucleus.

The immunoreactivity to MC5 observed within cells in the present study resembles that found in cells of the rat brain. Stichel and Singer [52] described two types of cells, based on the characteristics of PKC$\alpha\beta$ immunoreactivity: type I neurones with strong PKC$\alpha\beta$ immunoreactivity along the cell membrane and type II neurones displaying a more diffuse PKC$\alpha\beta$ immunoreactivity in the cytoplasm of the somata. In the present study, both of these types of cell-staining were observed. However, in chick brain, cells displaying PKC$\alpha\beta$ immunoreactivity along the cell membrane were S100-positive and NSE-negative. Therefore, they are probably glial cells, not neurones, embedded within the anterior commissure and scattered throughout the paleostriatal complex. The S100 protein and the coexpressed PKC in these glial cells may be linked functionally since it is known that S100 inhibits phosphorylation of PKCβI substrate proteins [49,51]. In the chick brain, cells which were immunoreactive to PKC$\alpha\beta$ and showed uniform staining of the cytoplasm were S100-negative. They may, therefore, be neurones and correspond to type II neurones described by Stichel and Singer [52] in the rat brain.

In rat brain, the expression of the γ subspecies of PKC develops postnatally [14,20]. 36G9 immunoreactivity is virtually absent in the rat forebrain during the first 5 postnatal days; it is clearly present by postnatal day 7 and the adult distribution is achieved by postnatal day 30 [14]. In chick brain, however, the expression of PKCγ is abundantly detectable with 36G9 at postnatal day 1. This early expression of PKCγ in chick brain might reflect the difference between precocial and altricial species since chicks are capable of walking and running within a few hours of hatching and are, of course, able to learn [6].

The differential distribution of the PKC isoenzymes raises the possibility that in the IMHV some axonal inputs containing PKC$\alpha\beta$ terminate upon postsynaptic cells expressing the γ form of PKC. The origin of the PKC$\alpha\beta$-positive fibre plexus found in the IMHV is not known. However, the hippocampus, septum and archistriatum contain PKC$\alpha\beta$-positive cell bodies which are S100-negative. These areas project to the IMHV [10,24]. The differential distribution of PKC isoenzymes in the IMHV has implications for an interpretation of the role of PKC in learning and memory. It is possible that PKC$\alpha\beta$, if present in axon terminals in the IMHV, plays a role in memory formation by being translocated from cytosol to the presynaptic membrane [12]. Postsynaptically, PKCγ activation may result in changes in the morphology of postsynaptic densities and/or changes in genomic activation. Interestingly, it has been reported that PKC activation by 4b-phorbol 12,13 diacetate in a slice containing the IMHV-produced changes in synaptic morphology similar to those found after imprinting [9]. Huang et al. [28] recently speculated that a pre- and postsynaptic differentiation of PKC isoenzymes underlies the selective sequential activation observed for the persistence of the plastic neuronal changes occurring during LTP. EM studies are neccesary to further elucidate the subcellular distribution of PKC isoenzymes in the chick brain.

Imprinting is associated with a significant increase in: (1) NMDA-sensitive binding of [^{3}H]L-glutamate to membranes prepared from the left IMHV, probably reflecting an increase in the number of NMDA receptors [37,38]; and (2) elevated levels in the IMHV of immunoreactivity for the Fos protein, the product of the immediate-early gene c-*fos* [39]. Nearly all Fos-positive neurones in the IMHV of chicks express PKCγ [1]. Thus, PKCγ is present in neurones that are likely to be involved in memory formation. PKC activity is required for the activation of immediate-early genes, such as c-*fos* [17], and modulates NMDA currents [3]; PKCγ may thereby play an important role in the mechanisms underlying imprinting. The differential distribution of PKC$\alpha\beta$ and PKCγ described in the present study may further clarify the understanding of the molecular processes underlying learning.

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